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Effect of Ascorbic Acid and Zinc Sulfate on Ethanol Toxicity and Metabolism[1] (39624)

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Normally, ethanol is metabolized at a constant rate in animals and man. The most successful attempts to accelerate disappearance of ethanol from the blood have been achieved with fructose (1-3) and thyroid hormone (4). However, a number of vitamins (5), amino acids (6, 7), and other nutrients (1) also has been tested with less impressive results.

Krasner *et al.* (8) have shown direct correlations between leukocyte ascorbic acid levels (a good index of total body ascorbic acid stores), the rate of clearance of ethanol from the blood, and the hepatic activity of alcohol dehydrogenase which is responsible for oxidation of ethanol to acetaldehyde and is recognized to be rate limiting in the metabolism of ethanol. However, subsequent work by the same authors has failed to demonstrate any specific *in vitro* effect of ascorbic acid on the activity of alcohol dehydrogenase (9) and no correlation was found between the concentrations of ascorbic acid and alcohol dehydrogenase activity in ascorbic acid-deficient guinea pigs (10).

Zinc is the metal component of alcohol dehydrogenase and plasma concentrations of this metal frequently are low in chronic alcoholics. Prasad *et al.* (11) have shown that hepatic alcohol dehydrogenase activity is decreased to about 60% of normal in zinc-deficient rats when compared to pair-fed control animals. The possibility that supplemental zinc might accelerate ethanol metabolism, perhaps by increasing hepatic activity of alcohol dehydrogenase, has not been tested previously.

Acute and chronic ethanol intoxication has been produced in rats and mice so that the effects of ascorbic acid and zinc supplements alone and in combination on survival and on ethanol metabolism could be examined. Both appear to have protective effects each, alone or in combination.

Methods. The first pilot study was designed to evaluate the chronic protective effects of ascorbic acid on ethanol toxicity in 250-g male Holtzman rats. Mortality rates were observed in four groups (five rats per group) given ip injections five times weekly for 4 weeks. The first group received 56 m*M* ethanol per kg body weight per injection; the second group, 0.56 m*M* ascorbic acid per kg body weight; the third group, an equivalent amount of both ethanol and ascorbic acid; and the fourth group, an equal volume of normal saline which was used as a diluent for both the ethanol and ascorbic acid.

The second series of studies was designed to determine the relative efficacy of ascorbic acid and zinc sulfate alone and in combination on mortality rates in 40-g Cf-1 Charles River female mice with induced ethanol toxicity. Group 2a studies were designed to evaluate the effects of different amounts of the trace metals zinc sulfate and manganese chloride on survival and to rule out any toxicity from the trace elements. Intraperitoneal injections of zinc sulfate (0.2 or 1.0 μM), manganese chloride (0.2 or 1.0 μM), or normal saline (0.5 ml) were given at zero hour. These treatment regimens were followed at 1 hr, 24 hr, and 7 days with ip injections of 4.1 m*M* ethanol. Nine or ten mice were placed in each treatment group. The number of surviving mice was counted on the tenth day.

Larger numbers of mice were studied in Group 2b. Thirty mice received 1 μM Zn^{2+} at zero hour and 35 received saline as a control. One hour later, 4.1 m*M* ethanol was injected ip and the survivors were counted 24 hr later.

In Group 2c, 20 animals were placed in each treatment group. Each of the groups of animals received one of the following alone

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or in combination: 25 mg of ascorbic acid, 0.2, 0.4, or 1.0 μM zinc as zinc sulfate, or 0.5 ml of normal saline. Ethanol injections (4.1 mM) were given at 1 and 24 hr after injection of the above, and the number of surviving mice was counted after 48 hr.

In the third series of studies, 40 450-g male Holtzman rats were divided into five groups. The first group received 2.5 mM ascorbic acid per kg body weight in 1.0 ml of saline; the second, 1.4 μM zinc as zinc sulfate per kg body weight in saline; the third, a combination of ascorbic acid and zinc; the fourth, 1.6 mM pyrazole per kg body weight in saline; and the fifth, 1.0 ml of saline as a control.

After 1 hr of equilibration, 15 mM per kg body weight of ethanol was injected ip in 1.0 ml of saline (40% solution). Blood samples were drawn at 30, 60, 90, 120, 150, and 180 min post-ethanol injection by first washing the tip of the tail with warm detergent water, rinsing it with distilled water, placing the tail under a heat lamp for 5 min, and thinly slicing off the tip of the tail. The first drop of blood was discarded and a heparinized capillary tube (100 μl) was allowed to fill with blood.

Blood ethanol determinations were performed with the gas chromatographic method of Jain (12). A gas–liquid chromatograph (Varian 1200) with Chromosorb W, AW, 60/80 mesh column was utilized for the ethanol analysis, and isobutyl alcohol was used as the internal standard. Equal volumes (50 μl) of both sample and standard containing known amounts of isobutanol were mixed thoroughly and a 0.5-μl aliquot was delivered with a 1-μl Unimetric syringe into the injection port and analyzed immediately. The nitrogen carrier gas flow was 35 ml/min; the oxygen flow, 100 ml/min; the hydrogen flow, 30 ml/min; the injection port temperature, 160°; and the oven temperature, 100°. Following each injection, the syringe was rinsed several times with the internal standard solution to prevent clogging of the needle. The ratios of ethanol to isobutanol in unknown and known standards were determined and utilized in a formula to determine ethanol concentrations in mg/100 ml (12). The sensitivity with this method is less than 1 mg/100 ml with a coefficient of variation of $\pm 5\%$.

Statistical analysis was accomplished with Student's t test for paired and unpaired data.

Results. In the initial pilot study, at the end of 4 weeks, there was 100% survival in the two groups receiving ascorbic acid alone or saline. None of the animals receiving ethanol alone survived. Four of five, however, survived in the group that received ethanol and ascorbic acid in combination.

In the second series of studies in mice, neither zinc sulfate (0.2 or 1.0 μM) nor manganese chloride (0.2 or 1.0 μM) produced any toxicity, as all animals receiving these trace metals survived. The results of the studies in the ethanol-intoxicated animals (4.1 mM) are summarized in Table I. In Groups 2a, only 3 of 10 animals pretreated with ethanol (control) survived the 10 days. Saline (control) survival rate was 100%. Pretreatment with 0.2 or 1.0 μM manganese chloride or 0.2 μM zinc sulfate had no effect on these survival rates. The survival rate in the group pretreated with 1.0 μM zinc was significantly better ($P < 0.05$) than the control group with six of nine animals surviving. In Group 2b, the mice pretreated with zinc sulfate followed in 1 hr by 4.1 mM ethanol showed a 24-hr survival of 77%. This was significantly better ($P < 0.05$) than the control group pretreated with ethanol where only 14% survived.

In Group 2c, only 13 of 40 mice (32%) injected ip with 4.1 mM ethanol on 2 consecutive days survived. In contrast, all 20 mice pretreated with 25 mg of ascorbic acid survived and 18 of 20 (90%) pretreated with zinc sulfate (1.0 μM) survived. Interestingly, smaller amounts of zinc (0.2 and 0.4 μM) improved the survival after the first injection but not after the second.

The third series of studies is illustrated in Fig. 1. Although the zinc- and/or ascorbic acid-treated animals had lower blood ethanol levels 30 min after ip injection of ethanol, these levels did not become significantly less ($P < 0.05$) until 60 min after injection. These differences persisted through the duration of the study. The slopes of the ethanol disappearance curves did not appear to be much steeper in the treated animals. In contrast, the animals

TABLE I. EFFECT OF PRETREATMENT WITH ASCORBIC ACID, ZINC SULFATE (Zn^{2+}), AND/OR MANGANESE CHLORIDE (Mn^{2+}) ON SURVIVAL RATES OF MICE INJECTED ip WITH 4.1 m*M* ETHANOL ON DIFFERENT SCHEDULES (1, 24, AND/OR 168 HR).

11	Number of mice	Treatment 0 hr	Ethanol (4.1 m*M*/ mouse) Times (hr)	Number alive 24 hr	48 hr	10 day	Survival (%)
Group 2a							
1	10	0.2 μM Zn^{2+}	1, 24, 168			3	30
2	9	1.0 μM Zn^{2+}	1, 24, 168			6	67*
3	10	0.2 μM Mn^{2+}	1, 24, 168			4	40
4	10	1.0 μM Mn^{2+}	1, 24, 168			3	30
5	10	Ethanol (control)	1, 24, 168			3	30
6	10	Saline (control)	1, 24, 168			10	100
Group 2b							
7	30	1 μM Zn^{2+}	1	23			77*
8	35	Ethanol (control)	1	5			14
Group 2c							
9	20	Saline (control)		20	20		100
10	20	Ethanol (control)	1, 24	6	6		30
11	20	0.2 μM Zn^{2+}	1, 24	20	4		20
12	20	0.4 μM Zn^{2+}	1, 24	20	2		10
13	20	1.0 μM Zn^{2+}	1, 24	20	18		90*
14	20	Ethanol (control)	1, 24	12	7		35
15	20	25 mg ascorbic acid	1, 24	20	20		100*
16	20	25 mg ascorbic acid + 1.0 μM Zn^{2+}	1, 24	16	16		80*

* Significant difference from controls, $P < 0.05$.

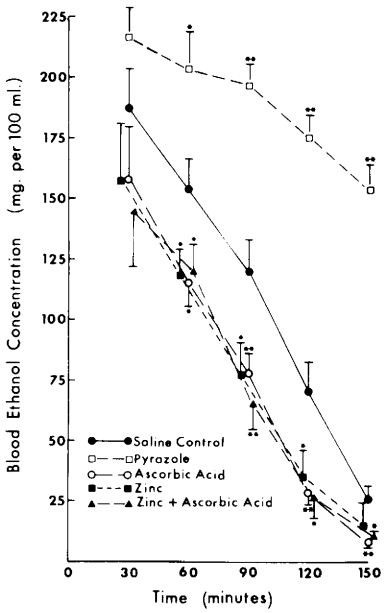

FIG. 1. Blood ethanol concentrations in mg/100 ml of treated groups (pyrazole, ascorbic acid, zinc, zinc and ascorbic acid) and saline control at 30, 60, 90, 120, and 150 min postinjection. Each point represents the mean ± SE of seven rats. Differences from control did not become statistically significant ($P < 0.05$) until 60 min after injection. * $P < 0.05$, ** $P < 0.01$, when treated groups were compared to control.

given pyrazole, an alcohol dehydrogenase inhibitor, had higher ethanol levels 30 min post-ethanol injection and had a much flatter ethanol disappearance curve.

Discussion. The first step in the hepatic elimination of ethanol is the oxidation of ethanol to acetaldehyde. This reaction is catalyzed mainly by alcohol dehydrogenase, an enzyme dependent upon nicotinamide adenine dinucleotide (NAD). Although alcohol dehydrogenase is recognized to be the major enzyme system responsible for oxidation of ethanol to acetaldehyde in the soluble fraction of the liver cell (13), two other systems also have been described, namely an oxidizing enzyme system present in the microsomal fraction of the cell (14) and the NADPH-dependent hydrogen peroxide which metabolizes ethanol to acetaldehyde via catalase (15). Further dehydrogenation of acetaldehyde to free acetate is accomplished by another NAD-dependent enzyme, aldehyde dehydrogenase. The first reaction is the slowest and, therefore, the rate-limiting step, and it appears to be dependent upon the capacity of the liver to reoxidize the NADH formed.

Pawan (1) studied the effects of various

vitamins and sugars on the rate of ethanol metabolism in man and reported that 600 mg of ascorbic acid given acutely had no influence on ethanol clearance rates. Krasner *et al.* (8) later reported a direct correlation existed between leukocyte ascorbic acid levels (an index of ascorbic acid stores), hepatic alcohol dehydrogenase activity, and the clearance of ethanol from the blood. Furthermore, they showed that administration of 1 g of ascorbic acid daily for 2 weeks raised leukocyte ascorbic acid levels and ethanol clearance rates in 9 of 11 patients. Our findings of increased survival and decreased blood ethanol levels after a standard ethanol load in animals pretreated with ascorbic acid partly support their initial observation which, in later publications (9, 10), they were unable to confirm.

Although ascorbic acid does not participate directly in the metabolism of ethanol to acetaldehyde to acetate, it is a strong reducing agent. The possibility exists that ascorbic acid can function as an electron donor similar to NAD in ethanol metabolism; hence, it spares the NAD/NADH and accelerates the conversion of ethanol to its metabolites.

Zinc is the metal component of the metalloenzyme, alcohol dehydrogenase. Prasad *et al.* (11) have shown that hepatic alcohol dehydrogenase activity is decreased to about 60% of normal in zinc-deficient rats when compared to pair-fed control animals. The effect of zinc supplementation in normal animals has not been reported. Furthermore, no previous studies have been reported on the effects of zinc supplements on ethanol clearance or survival rates in ethanol intoxication. Equimolar quantities of manganese chloride failed to provide similar protection, suggesting that this is an effect specific for zinc.

No additive or synergistic effect could be observed in providing protection against ethanol intoxication or in increasing ethanol clearance from the blood when the two agents (ascorbic acid and zinc sulfate) were given in combination. Whether these two agents provide protection against ethanol intoxication other than that related to more rapid clearance of ethanol from the blood remains undefined.

Although the blood ethanol concentrations 60 min after injection were significantly lower in the ascrobic acid- and/or zinc sulfate-treated rats, the slopes of the ethanol disappearance curves were not steeper thereafter, suggesting the metabolism of ethanol was not accelerated after 1 hr. Three possible explanations for this observation exist. The first is that ascorbic acid and zinc sulfate delay the absorption of ethanol from the peritoneum. Against this possibility is the failure to see evidence of delayed absorption of ethanol which should tend to flatten the disappearance curve. The second is that ascorbic acid and zinc sulfate increase the volume of distribution of ethanol. This is unlikely as ethanol normally distributes rapidly through all extracellular and intracellular fluid spaces.

The third possibility is that the ascorbic acid and zinc sulfate exert their effects on ethanol metabolism during the first hour, exhausting any effect thereafter. If the increased disappearance of ethanol from the blood were due to increased hepatic alcohol dehydrogenase activity, one would expect to see steeper ethanol disappearance curves in the pretreated animals. The clinical significance of these observations remains to be tested.

Summary. Acute (single dose) and chronic (multiple dose) ethanol intoxication has been produced in rats and mice so that the effects on survival and on ethanol metabolism could be determined in animals pretreated with ascrobic acid and/or zinc sulfate. Only 13 of 40 mice (32%) injected ip with a fixed amount of ethanol on 2 consecutive days survived. In contrast, the survival rates in matched animals pretreated with ascrobic acid (25 mg) or zinc (1 μM) were 100 and 90%, respectively (20 animals per group). Smaller amounts of zinc (0.2 and 0.4 μM) improved the survival after the first injection of ethanol (24 hr), but not after the second (48 hr). Similar observations were made in rats given repeated injections of ethanol over a 4-week period. Serial blood ethanol concentrations were determined in rats pretreated with ascrobic acid, zinc, a combination of ascorbic acid and zinc, pyrazole (an alcohol dehydrogenase inhibitor), or saline (control). Blood ethanol concentrations were significantly

lower ($P < 0.05$) 1 hr after ip ethanol injections in animals pretreated with ascorbic acid and/or zinc when compared to saline control animals. Pyrazole, in contrast, maintained increased blood ethanol levels. These studies indicate that both ascorbic acid and zinc exert protective effects in ethanol-intoxicated rodents.

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